Enhancing Effect of Cetyl Lactate on the Percutaneous Absorption of Indomethacin in Rats

Fusao KAIHO,* Reiko KOIKE, Hideaki NOMURA, Hiromi HARA, Kimiko MARUOKA, Masahiko DOHI and Yuriko KATO

Faculty of Pharmaceutical Sciences, Science University of Tokyo, Ichigaya Funagawara-machi, Shinjuku-ku, Tokyo 162, Japan.
Received October 24, 1988

The enhancing effect of cetyl lactate (CL) on the percutaneous absorption of indomethacin (ID) from test solutions in propylene glycol (PG) was investigated by using the abdominal skin of rats *in vivo*.

The percutaneous absorption rate of ID from 1 or 3% CL–PG test solution through the intact skin of rats was observed to be faster than that from the control solution (without CL). The bioavailability of ID was about 0.04% for the control solution, 2.2% for 1% CL–PG and 6.8% for 3% CL–PG test solutions. These results suggest that CL functions as an enhancer for the percutaneous absorption of ID. Furthermore, marked enhancing effects on percutaneous absorption of ID were obtained at a concentration greater than 1% CL in PG.

In order to elucidate the mode of action of CL as an absorption enhancer, the percutaneous absorption of ID from the control solution and 3% CL–PG test solution through damaged skin from which the stratum corneum had been stripped was additionally investigated. It was confirmed that CL acts on the stratum corneum to produce its effect.

Keywords cetyl lactate; propylene glycol; enhancer; indomethacin; percutaneous absorption; blood level

It is well known that the skin itself presents an effective barrier to topically applied drugs. It is thus not an easy matter to absorb a drug through the skin as compared with through the rectal or the oral mucous membrane.

One method to improve the bioavailability after the topical application of drugs is to employ a penetration enhancer. Many substances have been suggested as enhancers of percutaneous absorption; *e.g.*, dimethylsulfoxide, urea, decyl methyl sulfoxide, 2-pyrrolidone, 1-dodecylazacycloheptan-2-one (Azone), *N*,*N*-diethyl-*m*-toluamide, calcium thioglycolate, oleic acid, propylene glycol (PG), *etc.*[1] Furthermore, many investigations concerning the effects of these substances as enhancer *in vivo* or *in vitro* have been carried out.

Nevertheless, little attention has been paid to cetyl lactate (CL), which is used as an ingredient of cosmetics such as lipstick, milk lotion, cream, hair tonic and aftershave lotion.[2] CL is particularly used as a coupling agent for oily constituents of lipstick.

In the present study, we investigated the influence of CL on the percutaneous absorption of indomethacin (ID), selected as a model drug. The efficiency of percutaneous absorption of ID was determined by measuring the drug concentration in rat plasma.

Experimental

Materials PG was purchased from Tokyo Kasei Co., Ltd. CL was kindly supplied by Musashino Chemical Laboratory Ltd. ID was purchased from Sigma Chemical Company. All the solvents used in this experiment were of reagent grade from Kanto Chemical Co., Ltd.

Preparation of Test Solution The test solutions used for this study were prepared according to the formulae in Table I. CL and ID were dissolved in pure PG with the aid of heat (about 50 °C). The content of ID was controlled so as to give 1% in each test solution.

Animals Male Wistar rats weighing between 230 and 250 g were used in this study. These rats were supplied by Nippon Rat Co., Ltd, Saitama, Japan.

Treatment of Rat Abdominal Skin The intact skin and the damaged skin were obtained as follows. In the case of intact skin, the hair of the abdominal region was carefully removed with an electric hair clipper and an electric razor without breaking the skin. To obtain damaged skin, the hair of the abdominal region was cut and removed, then the stratum corneum was removed by 20 successive strippings using cellophane adhesive tape.

***In Vivo* Experiment** A rat was fixed on its back and a glass chamber ($27\,cm^3$) was applied to the surface of the treated abdomen ($3 \times 6\,cm^2$) using a surgical tissue cement (Aron Alpha, Toa Gosei Chemical Co., Ltd.). Then 10 ml of test solution was placed in the chamber. Blood samples (0.3 ml) were withdrawn from the jugular vein into a syringe at predetermined intervals, and were centrifuged at 3000 rpm for 10 min. The resulting plasma samples (0.1 ml) were removed for analysis of ID by high performance liquid chromatography (HPLC).

TABLE I. Formulae of Test Solutions

Composition[a]	Test solution No.[b]			
	1[c]	2	3	4
Cetyl lactate	—	0.5	1	3
Propylene glycol	100	99.5	99	97

a) In grams. *b*) The content of ID was fixed at 1% in each test solution. *c*) No. 1 test solution is the control solution in this paper.

Assay of ID In the *in vivo* experiment, the HPLC method was applied for measurement of ID. A plasma sample (0.1 ml) was placed in a test tube (5 mm i.d. × 50 mm) and 0.5 ml of internal standard (flufenamic acid, 10 μg/ml) acetonitrile solution was added. After being shaken for 3 min, the mixture was centrifuged at 3000 rpm for 10 min. The upper layer was transferred to another test tube, and was concentrated to about one-twentieth of its original volume under a stream of nitrogen. A 30 μl aliquot of this solution was injected into the HPLC apparatus (SSC-3100, Senshu Scientific Co., Ltd.). The conditions for analysis were as follows: column, SSC-ODS-262 (Senshu Scientific Co., Ltd.); mobile phase, 0.01 M sodium acetate buffer (pH 3.2)–methanol (3:7, v/v); flow rate, 2.0 ml/min; detector, ultraviolet (260 nm). The signal from the detector was fed into an integrator (Shimadzu Chromatopack E-1A, Shimadzu Seisakusho Co., Ltd.).

Results and Discussion

Percutaneous Absorption of ID from Test Solutions The mean semi-log plasma ID concentration–time curves after the topical administration of four test solutions are shown in Fig. 1. In addition, the area under the plasma concentration–time curves (*AUC*s) and the bioavailabilities are listed in Table II.

After application of No. 4 test solution (containing 3% CL in PG), rapid elevation of the plasma ID concentration was observed as compared with the other test solutions. Furthermore, the mean *AUC*, the bioavailability and the mean ID plasma concentration for 8 h after administration of No. 4 test solution were also statistically significantly

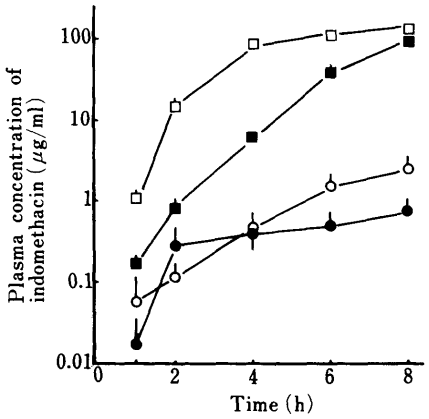

Fig. 1. Effects of Cetyl Lactate on the Percutaneous Absorption of Indomethacin in Rats

●, No. 1 test solution (PG alone); ○, No. 2 test solution (0.5% CL in PG); ■, No. 3 test solution (1% CL in PG); □, No. 4 test solution (3% CL in PG). Each value represents the mean ± S.E. of five experiments.

TABLE II. Area Under the Plasma Concentration–Time Curve (*AUC*) and Bioavailability after Topical Administration of Test Solutions to Intact Skin of Rats

	Test solution No.			
	1	2	3	4
AUC_{0-8} (μg·h/ml)	3.9 ± 1.3[a]	5.8 ± 3.0[a]	187.8 ± 29.4[b]	581.1 ± 59.4
Bioavailability[c] (%)	0.04 ± 0.02[a]	0.08 ± 0.04[a]	2.21 ± 0.35[b]	6.84 ± 0.70

Each value represents the mean ± S.E. of five experiments. *a*) Statistically significant difference from Nos. 3 and 4 test solutions ($p<0.01$). *b*) Statistically significant difference from No. 4 test solution ($p<0.01$). *c*) Bioavailability (%) = $(AUC_{p.c.} \times dose_{i.v.}/AUC_{i.v.} \times dose_{p.c.}) \times 100$ where $AUC_{p.c.}$ and $AUC_{i.v.}$ are *AUC* after percutaneous and intraveneous administrations, respectively.

higher than those of other test solutions. The percutaneous absorption rates of ID from Nos. 1 (without CL; control) and 2 (containing 0.5% CL in PG) test solutions were considerably slower than that from No. 4 test solution. The ID plasma level of about 150 μg/ml for No. 4 test solution at 8 h was in particular contrast with that of about 0.8 μg/ml for the control solution. The percutaneous absorption pattern of ID after the application of the control solution was observed to be similar to that of No. 2 test solution. The mean plasma concentrations in the case of No. 2 test solution at 6 and 8 h after administration were higher than those in the case of the control solution, but the differences were not significant. Furthermore, the *AUC* and the bioavailability of No. 2 test solution were similarly higher than those of the control solution, but without statistical significance. The percutaneous absorption of ID from No. 3 test solution (containing 1% CL in PG) was lower than that from No. 4 test solution, but was still markedly greater than that from the control or No. 2 test solution. The mean ID plasma concentrations at 2, 4, 6 and 8 h, as well as the *AUC* and the bioavailability of No. 3 test solution were significantly higher than those of the control and No. 2 test solutions after administration.

Thus, the addition of CL to PG was observed to enhance the percutaneous absorption of ID through rat skin as compared with the control solution. A marked enhancing effect was obtained at a concentration greater than 1% CL in PG within 8 h after administration. Unfortunately, CL has a narrow concentration range of application as an percutaneous absorption enhancer for ID, because the solubility of CL in PG at room temperature was found to be as low as 3%.

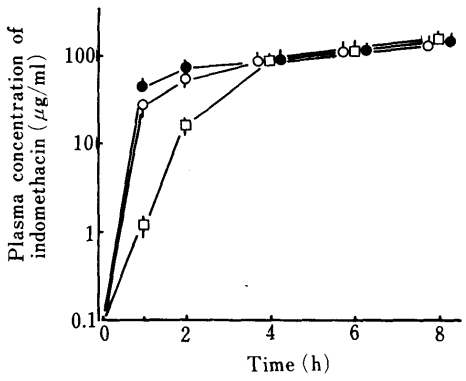

Fig. 2. Percutaneous Absorption of Indomethacin through the Damaged Skin of Rats

●, No. 1 test solution (PG alone); ○, No. 4 test solution (3% CL in PG); □, No. 4 test solution through the intact skin (from Fig. 1). Each value represents the mean ± S.E. of five experiments.

PG itself has been considered as an enhancer for the absorption of drugs through the skin for a long time; *e.g.*, Barrett *et al.* reported that the incorporation of PG into a vehicle enhanced the percutaneous absorption of fluocinolone acetonide.[3] In addition, it has been reported that the addition of an absorption enhancer, such as Azone or oleic acid, to PG resulted in marked promotion of the absorption of an active ingredient through the skin as compared with the case of using PG alone (without enhancer).[4,5] The increase of percutaneous absorption of an active ingredient in a vehicle system containing Azone and PG is thought to be due to a synergistic action by Azone and PG, though the details have not been established. The increase of percutaneous absorption of ID by CL is also assumed to be due to a synergistic effect of CL and PG.

Percutaneous Absorption of ID from Test Solution through Damaged Skin of Rats Figure 2 shows the mean plasma concentration of ID after the topical administration of the control and No. 4 test solutions to the damaged skin of rats, from which the stratum corneum has been stripped. The mean plasma concentration of ID for 8 h following the administration of No. 4 test solution to the intact skin (already shown in Fig. 1) is included in Fig. 2 for comparison.

As shown in Fig. 2, there was no significant difference in the absorption patterns and the mean plasma concentration of ID between the control and No. 4 test solutions for 8 h after application to the damaged skin. The initial percutaneous absorption rates of ID after the topical application of the two test solutions through the damaged skin increased rapidly compared with that through the intact skin. In addition, the mean plasma concentrations of ID through the intact skin at 1, 2 and 3 h were significantly lower than those in the case of the damaged skin ($p<0.01$). However, no difference were detected in the mean plasma concentration of ID between the intact and the damaged skin at 4, 6 and 8 h after the application of No. 4 test

solutions.

These results show that CL acts on the stratum corneum. Cooper has reported that the fluidity of the lipid layer of the stratum corneum was increased by the topical application of certain two-component systems such as oleic acid–PG, and its barrier function was considerably reduced.[5] A similar effect may operate in the case of CL–PG for enhancing the absorption of ID through the skin.

In order to elucidate in detail the enhancing effects of CL and other fatty alcohol–lactic acid esters, such as lauryl lactate and myristyl lactate, on percutaneous absorption of drugs, further experiments are in progress.

References

1) *a*) J. P. Astley and M. Levine, *J. Pharm. Sci.*, **65**, 210 (1976); *b*) J. Beastall, R. H. Guy, J. Hadgraft and I. Wilding, *Pharm. Res.*, **3**, 294 (1986); *c*) E. Touition, *Int. J. Pharmaceut.*, **43**, 1 (1988); *d*) K. S. Ryatt, J. M. Stevenson, H. I. Maibach and R. H. Guy, *J. Pharm. Sci.*, **75**, 374 (1986); *e*) J. W. Wiechers, B. F. H. Drenth, J. H. G. Jonkman and R. A. de Zeeuw, *Pharm. Res.*, **4**, 519 (1987); *f*) K. Sugibayashi, K. Hosoya, Y. Morimoto and W. I. Higuchi, *J. Pharm. Pharmacol.*, **35**, 578 (1985); *g*) J. J. Windheuser, K. L. Haslam, L. Caldwell and R. D. Shaffer, *J. Pharm. Sci.*, **71**, 1211 (1982); *h*) K. Kushida, K. Masaki, M. Matsumura, T. Ohshima, H. Yoshikawa, K. Takada and S. Muranishi, *Chem. Pharm. Bull.*, **32**, 268 (1984); *i*) M. Yamada, Y. Uda and Y. Tanigawara, *ibid.*, **35**, 3399 (1987); *j*) B. W. Barry, *J. Controlled Release*, **6**, 85 (1987).
2) T. Tamura, "Koushouhin Kagaku," Nippon Mouhatsu Kagaku Kiyoukai, Tokyo, 1977, p. 106.
3) C. W. Barrett, J. W. Hadgraft, G. A. Caron and I. Sarkany, *Br. J. Dermatol.*, **77**, 576 (1965).
4) *a*) N. V. Sheth, D. J. Freeman, W. I. Higuchi and S. L. Spruance, *Int. J. Pharmaceut.*, **28**, 201 (1986); *b*) P. K. Wotton, B. Møllgaard, J. Hadgraft and A. Hoelgaard, *ibid.*, **24**, 19 (1985); *c*) M. Yamada and Y. Uda, *Chem. Pharm. Bull.*, **35**, 3390 (1987).
5) E. R. Cooper, *J. Pharm. Sci.*, **73**, 1153 (1984).